DEVELOPMENT OF A PORTABLE DNA SENSOR SYSTEM

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ABSTRACT

A fully-integrated, palmtop, field-portable DNA analyzer that is capable of responding within 30 minutes to a sample of as little as three strands of DNA will enhance the survivability of the warfighter. This technology has been sponsored through the Institute of Collaborative Biotechnologies and was lead by Nanex LLC with co-investigators from the US Army Research Laboratory and the University of California, Santa Barbara. This analyzer will not sacrifice selectivity or operational convenience. Reliable, high-speed detection of DNA will provide broad support in many Army missions. This project will transition findings and breakthroughs derived from UARC basic research programs to a device that can **Detect to Protect** by performing environmental sampling for biological threats. The analyzer will be able to monitor indigenous foods supplied to deployed troops by detecting inherent or intentional contaminants including native microorganisms, typical food-borne pathogens and biowarfare agents. As more is learned about physical changes during infection, it can **Detect to Treat** through use in medical diagnostics.

Current technology informs soldiers when to take a protective posture against chemical threats. This sensor will provide an analogous technology for biological threats. The potential functionality, low cost, ruggedness, ease of use and portability of this analyzer will encourage broad deployment and use, greatly enhancing the safety of troops in times of peace and at war.

1. INTRODUCTION

This paper describes the development of a portable analyzer capable of performing electrochemically-based bioassays and molecular diagnostics. The system is able to detect extremely low concentrations of DNA and RNA through the use of DNA amplification and without the use of optics or high voltages and with minimal use of reagents. The analyzer incorporates: 1) disposable lab-on-chip with electrochemical sensor, 2) instrument amplifier, control, and interface electronics.

The sensor will incorporate on a single substrate both target amplification and the biosensing capabilities of electrochemical DNA (eDNA) technology. Component integration will reduce the size, logistics load, and power requirements. This utilizes the reagentless detection capabilities of eDNA technology recently developed at UCSB and continue to pursue optimization of the response time, reusability and robustness of the sensor.

2. TECHNOLOGY DEVELOPED

eDNA is an electrochemically interrogated nucleic acid hybridization-based biosensor platform. A specific sequence of stem-loop ssDNA capture strand is immobilized on a gold electrode along with a coadsorbate to provide stability and reduce nonspecific binding. Upon interaction with the complementary strand of DNA or RNA from the target the stem-loop structure opens and quenches the redox signal from the label covalently bound to the capture strand (Figure 1). The sensor can be regenerated for repetitive use by rinsing with deionized water. (Xiao, 2006; Sumner 2006)

2.1 Optimization of the eDNA sensor

Considerable effort was focused on optimization of the signaling properties of the sensor platform by characterization of the parameters that determine the response of the functionalized electrodes. The eDNA sensor was demonstrated suitable for PCR product detection,

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insensitive to likely interferants, while improving signaling shelf life and specificity.

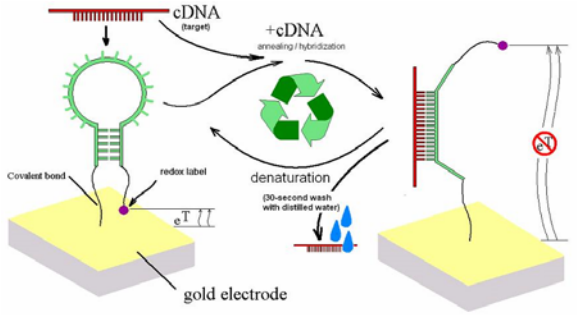

Figure 1. Electrochemical version of a surface bound molecular beacon (E-DNA).

2.1.1 Detection of a Single Point Mutation

Discrimination of a single base pair mismatch was achieved for more robust pathogen detection. Improvements in stability resulted from use of longer-chain alkanes in the SAMS. Sensors built using C-11-linked DNA probes (as opposed to C-6 linkers) are relatively thermostable, allowing us to perform eDNA detection at elevated temperatures and the hybridization thermodynamics of DNA are such that increasing temperature increases discrimination. For example, whereas at room temperature there is only a ~5% difference in peak height upon challenge with a single-base mismatch, at 50°C there is a more than two-fold reduction in peak height upon the introduction of a mismatch (Figure 2).

Moreover, a secondary benefit of sensing at elevated temperatures is improved equilibration time. Whereas the observed sensor response to a 50 base target equilibrates with a time constant of ~12 minutes at room temperature, at 50°C the equilibration time constant is significantly less than 5 minutes. This is a vast improvement, and the simple expedient of adding a heating element to the sensing chambers significantly improves the sequence specificity and speed of detection. The sensors were tested against a wide range of potential contaminants and specificity and reusability were found to be unaffected. Potential interferants tested include a wide variety of foodstuffs such as whole milk, coconut milk, infant formula, etc., and clinical materials such as blood serum, urine, etc. The sensors were also successfully tested against a wide range of single and multiple mutant DNAs. (Lubin, 2006)

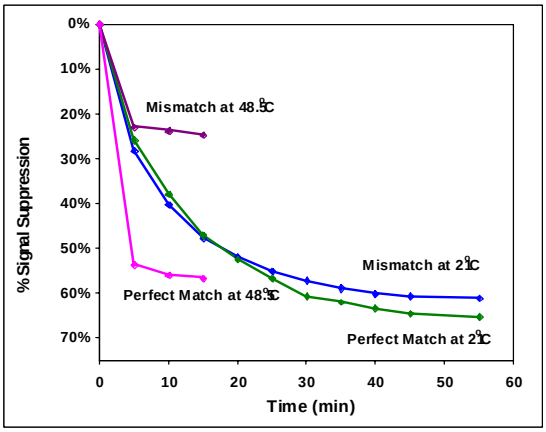

Figure 2. Sensor performance showing the discrimination of perfect match versus single base pair mismatch at room temperature ~50 Celsius.

2.1.2 Enhanced Signal Gain Via Control of ACV Frequency

eDNA signaling is a result of hybridization-induced changes in the dynamics of the eDNA probe. The dynamic factor is the rate with which the appended redox label strikes the electrode surface. This was deduced from the results of studies of eDNA signal gain versus the density of the eDNA probes on the sensor surface.

eDNA signal gain may be modulated by controlling the frequency of the AC current used to interrogate the sensor. Our rationale: at very low AC frequencies both the relatively rigid target-probe duplex and the unbound stem-loop probe have sufficient time to collide with and transfer electrons to the electrode. In contrast, at higher frequencies only the stem-loop structure can transfer electrons fast enough to respond to the AC current. Thus there is an increased sensor gain at higher frequencies over that observed at lower frequencies. Experiments conducted over a range of AC frequencies and probe densities show no detectable gain at low AC frequencies and significantly enhanced gain at higher frequencies.

Previously, probe density was viewed as a trade off: higher densities lead to improved gain, but greatly slowed sensor equilibration time. By employing higher AC probe frequencies (100s of Hz) maximal signal gain will be approached without sacrificing sensor response time. (Ricci, 2007)

2.1.3 Probe Density Easily Controlled

An alteration in the packing of the probes on the sensor surface has a significant effect on the signaling properties of the sensor platform. The effect of probe density on signal gain, specificity and sensor equilibration time was explored and optimal values determined. The density is simply the ratio stem-loop probes per unit area of electrode surface. This is directly relevant to our ability to consistently fabricate eDNA sensors with a given probe surface density.

Probe density was controlled by changing the concentration of probe DNA employed during sensor fabrication. Using this approach eDNA electrodes with probe densities ranging from 6.5×10^{-14} to 3.5×10^{-12} mol/cm^2 (corresponding to packing of 3.9×10^{-6} to 2.1×10^{-4} molecules/Å^2) were reproducibly fabricated by employing probe DNA concentrations of 0.005 to 5 μM during fabrication. Attempts to fabricate sensors with lower probe densities fail to produce stable, active films and no electrochemical signal is detectable.

2.1.4 Enhanced Signal Gain and Equilibration Time Via Control of Probe Density

Previous studies using linear DNA probes suggest that the hybridization efficiency of surface-attached DNA molecules decreases monotonically with increasing probe density. The probe-density-dependence of eDNA signaling is more complex. For example, the highest probe densities investigated produced the *largest* signal suppression (~71% at our test target concentration). As probe density is reduced the observed signal suppression decreases before reaching a plateau value of ~41%. The effect of probe density on sensor specificity was tested using partially mismatched targets. The ratios of suppression obtained with the fully complementary target to that observed with mismatched targets provided a measure of the sensor specificity. These ratios are indistinguishable among sensors representing low, medium and high probe densities, indicating that eDNA specificity is not a strong function of this parameter.

Although signal suppression is optimal at higher probe densities, the equilibration times are also increased. The equilibration time constants we observed for high density sensors (~40 min.) are two to eight times longer than the equilibration time constants of medium and low density sensors respectively. This difference appears to arise due to the accessibility of the target sequence to the DNA probe. High-density sensors are characterized by a relatively densely packed probe layer, which likely hinders target accessibility and limits the rate of target-probe hybridization.

2.1.5 Understanding the Mechanism of Electron Transport

Understanding the mechanisms of electron transport from the redox label directly relates to signal generation, background noise, the effects of packing density, and other critical mechanisms. The electron transfer rate depends on the conformation of the DNA, with stem-loop DNA supporting a high transfer rate. That rate is limited by the intrinsic electron transfer rate of the redox moiety. The target-probe duplex supports only a slow transfer rate, limited by the rate of collision of the redox label with the electrode.

Sensor data collected using both methylene blue and ferrocene were very similar in both redox response changes upon hybridization and storage. While we feel either label would be suitable for sensor development ACV results using ferrocene exhibit flatter baselines. Also ferrocene's single electron transfer reaction makes a probe that is more easily modeled. Therefore, electron transfer rates were measured in the presence and absence of target using ferrocene-modified probes.

The measurements and model estimates electron transfer rates from plots of the ratio of the peak current to the background current. The plot of the ratio of peak to background currents versus AC frequency exhibits a dramatic change in slope as electron transfer is no longer rapid enough to keep pace with the rapidly oscillating applied potential. The electron transfer rate observed for a low-density sensor slows by an order of magnitude upon target binding (from ~20,000 to ~2,000 sec^{-1}), presumably reflecting the collision-limited transfer rate of the hybridized probe-target duplex. In contrast, the observed electron transfer rate for high-density sensors, ~20,000 sec^{-1}, remained unchanged upon hybridization. This presumably occurs

because, under these conditions, transfer from the hybridized probe-target duplex is blocked that transfer from of the remaining, unhybridized stem-loop probe molecules is observed.

It had previously been speculated that, upon hybridization, the electrochemical signal generated in eDNA-like sensors resulted from an "electron tunneling effect" along the double helix, so that the signal suppression observed arose solely due to the increased tunneling distance between the redox moiety and the electrode. However, data collected in this program suggests hybridization induces changes in the rate with which the redox moiety collides with the electrode surface. At low probe densities, the signal suppression observed upon hybridization is limited because even hybridized probe DNA can, on occasion, collide with the electrode surface and transfer electrons. At high probe densities, in contrast, the steric hindrance of the closely-packed probe DNA precludes these collisions, leading to higher signal suppression despite presumably lower hybridization efficiency. (Ricci, 2007)

2.1.6 eDNA Compatibility with PCR

eDNA-based detection of PCR products in PCR buffer was characterized for specificity and kinetics. Under these conditions sequence-specific discrimination can be performed between amplicons from the *gyraseB* gene of *E. coli* and two *Salmonella* strains. As was true for the detection of 17 base oligonucleotide targets, eDNA sensors directed against terminal sequences in longer (100 base) amplicons exhibit signal saturation within the 5 minute dead time of current experiments. The response time constant for internal sequences centrally located within a 100 base element are much slower and only achieve saturation after ~40 minutes. The total signal changed for a given target concentration is also slightly smaller (47% versus 52%). Nevertheless, more than half of the total signal change is observed within the 5 minute dead time of current experiments, suggesting that eDNA signal generation will not significantly slow the response time of PCR/eDNA chips. This work was published in *Proc. Natl. Acad. Sci*. (Lai, 2006)

2.1.7 Improved Sensor Shelf Life

The shelf-life of functionalized, on-chip electrodes has been dramatically improved over this program. Upon drying for storage and re-wetting for use the original, C-6-thiol linked sensors would lose approximately 20% of their initial signal strength. C-11-thiol linked to phosphoramadites were synthesized for the direct incorporation of long-chain alkane thiols into DNA during synthesis. Using this approach and improved annealing conditions eDNA sensors were fabricated using these long-chain alkanes and found their stability and shelf-life to be very dramatically improved.

The new sensors withstood eight rounds of heating to 95°C (*i.e.* the harshest temperatures employed in PCR) and storage under buffer for more than five days with only modest signal degradation of either the initial signal or the signal change upon target binding (Figure 3). Similarly, test results on the new sensors are within 2% of their initial signal after drying, storing them at room temperature for several days and rehydrating them.

The SAM modifications significantly improved sensor reusability. Whereas the original sensor architecture achieved only 80% signal recovery the sensor now achieves > 99% signal recovery even when the sensor is challenged in "dirty" samples such as blood serum (Figure 3). (Lai, 2006; Seferos, 2006)

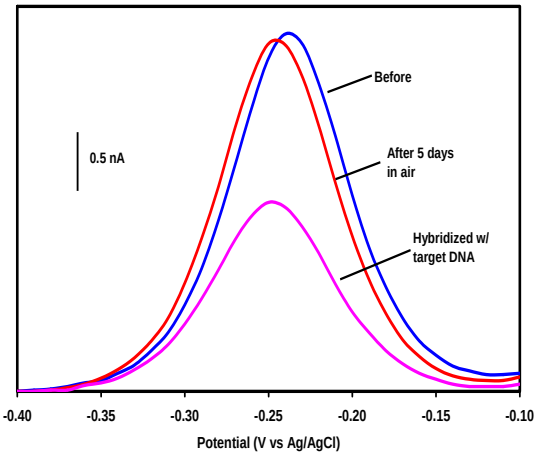

Figure 3. Sensor performance after storage followed by rehydration and interrogation.

2.1.8 Method for Independently Coating of Multiple Electrodes *in situ*

An electrochemical method for the *in situ* differential labeling of the working electrodes was developed based on the use of a potential sweep between 0.725V and 0.75V to

selectively desorb thiol layers from a single electrode. This method allowed the attachment of two different probes to two of the sensing pixels. Subsequently, targets against two serotypes of the influenza virus, H1N1 and H5N1, were detected. This work was published in *Langmuir*. (Lai, 2006 PNAS; Ricci, 2008)

2.2 Development of Robust Electrodes

Titanium (Ti) was used as an adhesion layer to create a strong interface between the Pt and the passivation layer. After thermal bonding of the glass layer containing the electrodes with the cover glass no cracks were visible in the Pt. The cracking problem was solved by including a Ti layer to improve bonding and relieve stress at the interface between layers of material with different coefficients of thermal expansion.

The electrode fabrication process is as follows. Photo resist (PR) was used to pattern all layers. Evaporation deposition was used to create a 200 Å layer of Ti on a glass substrate. This layer Ti - and all Ti layers to follow - was designed to create a strong bond and act as athermal buffer layer. An 1800 Å layer of Pt was deposited to form the electrical traces and the base for the gold electrodes. A 200 Å layer of Ti was deposited over the portion of the Pt to be coated with SiO_2. A 4000 Å layer of SiO_2 was sputtered over this. The SiO_2 is to insulate the Pt traces from the analyte solution. A 200 Å layer of Ti was then deposited over the areas of Pt designated as the working electrodes and to be coated with gold. This was followed by an 1800 Å layer of gold deposited to form the working electrodes. The counter and reference electrodes were left as bare Pt and not coated with the Ti or Au.

2.3 Multi-analyte detection

2.3.1 On-Chip eDNA Detection of the Salmonella *gyrB* Gene

Detection of the Salmonella *gyrB* gene was performed in the all-glass eDNA device using a pure DNA sample containing the ST 25 fragment of the *gyrB* gene. The eDNA chip was connected to a CHI Electrochemical Workstation in a 3-electrode set-up, i.e. a working electrode, a counter-electrode and a reference electrode. The electrochemical reactions were monitored using the CHI 730b software. The three sensing electrodes were cleaned by oxidoreduction in H_2SO_4 0.1 M. The probe DNA was immobilized, and the SAM layers were prepared in-situ. Then the target DNA at 400 nM was introduced and reduction scans were acquired after 5, 10 and 17 minutes hybridization time. Reduction scans were also acquired after the sensing electrode was regenerated using 1.5 ml DI water and filled with SSC×6 buffer.

The glass eDNA sensors behaved as designed and provided a very clean signal. In addition, the reduction scan's baseline remained approximately constant and level during the entire experiment. The high quality of the signal was due to the proper in-situ attachment of the DNA probe and passivation of the alkanethiols to the sensing electrodes.

2.3.2 Successful Test of eDNA Sensor Array

eDNA sensors were used to detect the Salmonella *gyrB* gene on three working electrodes. This simultaneous detection in 3 parallel electrodes within the eDNA device was a significant preliminary step towards multi-analyte detection. This was followed by an experiment with simultaneous detection of the avian flu virus (H5N1) and the human flu virus (H1N1) using two electrodes in a single cell (Figure 4). H5N1 is an RNA virus so the DNA probes were obtained via reverse transcription.

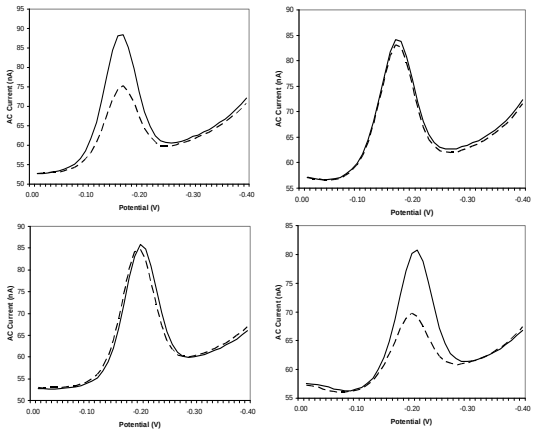

Figure 4. Demonstration of identification and discrimination of closely related viruses in same integrated eDNA chip. A) H5 target and probe, B) H5 target and H1 probe, C) H1 target and H5 probe, D) H1 target and probe.

The cyclic voltammetry curves resulting from the preliminary cleaning of the electrodes

revealed all three working electrodes were conductive and stable redox signals were observed. The current amplitude measured during DNA detection provided a readily detectable signal decrease during hybridization of the DNA probe to the target. The consistency of the results indicated a suitable electrode fabrication process and effective use of multiple electrodes and multiple sensors simultaneously.

2.4 Integration of PCR and eDNA

PCR and eDNA modules were integrated monolithically on a chip to perform genetic amplification, enzymatic single-stranded DNA (ssDNA) generation and detection of salmonella gyrase B amplicons from low initial copy number (50fM). After the PCR amplification, ssDNA is generated from double stranded (dsDNA) PCR products through Strandase exonuclease digestion of PCR products within the device. Finally, the sequence specific detection of ssDNA products is achieved via the on-chip eDNA assay which yields the electronic current signal.

2.4.1 Fabrication of PCR and eDNA Modules

The PCR module possesses an internal volume of 50μL and is designed to be robust and simple to fabricate. The chip itself is comprised of a PDMS channel sandwiched between two glass wafers. The glass wafers are cleaned and coated with 100nm of silicon dioxide to reduce non-specific adsorption of polymerase and thereby improve PCR yield. One of the glass wafers is then drilled with a CNC mill to provide inlets and outlets. The PDMS channels are formed by cutting the prescribed design into commercially available 250μm PDMS sheets with a precision electronic sign-cutter tool. The PDMS surfaces are made hydrophilic by a 5 minute UV-ozone treatment. The PDMS is then applied to the glass wafers, yielding a permanent bond. Finally, eyelets are fixed to the chip surface over the inlet/outlet holes, completing the device. The PCR device does not require heaters and temperature sensors as may be simply placed upon a commercially available thermofoil pad and temperature sensor (Minco, MN) powered via a commercial controller (Omega, CT).

In order to approach full device integration, the modules were first tested separately. Both PCR and eDNA devices were shown to operate successfully. After successful independent testing, the PCR and eDNA modules were integrated in a hybrid manner through external capillary tubing and 3-way valves. The eDNA module was shown to successfully detect synthetic salmonella target DNA following the PCR/Strandase process. (Pavlovic, 2008)

2.4.2 Monolithic Integration

Combining the eDNA and PCR modules onto a single substrate required significant design modification. Four critical factors needed to be addressed: (1) protection of eDNA monolayer from thermal damage resulting from PCR thermocycling, (2) uncompromised temperature control in the PCR region during thermal cycling, (3) effective mixing of product with Strandase and $MgCl_2$, (4) eDNA operation in PDMS.

A monolithic chip was designed capable of overcoming these issues. Issue (1) was solved readily by incorporating a small thermoelectric cooler (TEC) into the chip mounting apparatus. Maintaining a heating pad which covered only the PCR/Strandase chamber and positioning the TEC coplanar with the heating pad but covering only the eDNAchamber, allowed for the eDNA chamber to remain held at 18°C at all times during thermocycling and Strandase incubation. Furthermore, this arrangement did not impede amplification of PCR, satisfying issue (2).

To address effective mixing (3), narrow T-junctions leading to external an external mixing tube were incorporated to facilitate rapid diffusion of Strandase into PCR product and $MgCl_2$ into single-stranded product. Finally, to permit integration onto a single substrate, the relatively complicated eDNA fabrication procedure was supplanted by PCR/Strandase method (4). This meant that like the PCR/Strandase chamber, the eDNA chamber would be PDMS sandwiched between glass rather than a fusion-bonded all-glass chamber. Before assembling the device, the electrode wafer was cleaned thoroughly with piranha solution and the monolayer was immobilized on the electrodes using droplets of solution. From here, the device could be assembled and immediately ready for operation.

The new method of sensor preparation was shown to be effective in responding to

synthetic target DNA at various concentrations, yielding as low as 12% signal suppression at 250nM, which places it well within the range of PCR/Strandase output concentration. Moreover, the signals boast extremely low noise as demonstrated by an average relative standard deviation of 0.5%.

Finally, the entire chip has been shown to detect the presence of salmonella DNA at ~5fM initial template concentration (Figure 5). It does so through, PCR amplification from low initial template concentration, single-strand generation and eDNA detection.

Figure 5. Photograph of integrated PCR / Strandase and eDNA microfluidic chip. This chip uses external fluid reservoirs, heaters and pumps.

2.5 Circuitry and software

The analog potentiostat circuitry as well as the digital backend and software readout and display have been developed. Additionally these and control system for the PCR based unit have been tested.

2.5.1 Ruggedized PDA

TallaTech, the maker of the ruggedized PDA chosen by the Army, completed their first production run and delivered one of their new Model 57 ruggedized PDA (RPDA) devices for inspection. This device, in a former iteration, was known as the commander's digital assistant (CDA). We proceeded with development using a standard PC for the user interface while retaining the ability to port the software to a PDA at some future point. The RPDA has a standard universal serial bus (USB) port and thus development made using a PC will be easily ported.

2.5.2 Custom electronics PCBs

Digital readout circuitry has been developed using an FPGA for timing generation as well as control of the onboard A/D and D/A channels. This board also has a USB interface and currently supports 4 sensor channels. With software configuration modifications the design could support 16 or more channels.

Hardware and software control interface have been developed and built for PCR control. This hardware and software controls the full PCR system including temperature readout and control for both heating and thermoelectric cooling, valve actuation and syringe control.

The prototype circuit boards are larger than the size expected for a final product (Figure 6). Current platforms use discrete components and provide spare circuitry with wide operating ranges. As understanding progresses, it will be possible to narrow specifications and migrate to integrated circuits to significantly decrease the size and power requirements of the circuitry.

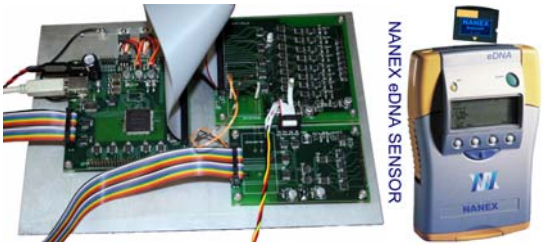

Figure 6. Control electronics and potentiostat PCB assemblies (left). Concept for future, integrated eDNA biosensor.

2.5.3 Multi-Channel Potentiostat

The critical analog circuitry needed to readout the eDNA target have been developed and tested for a 4 channel unit. This device may scale to a larger number of channels in the future as needed. This is critical in developing background noise subtraction techniques that will enhance the device sensitivity.

There were two aspects to this work: the first was the development of a potentiostat specifically for the eDNA sensor and the second was development of high-speed electronics useful for multi-element sensor arrays. In addition to these two specific areas, other methods for modulating and otherwise reducing the effects of drift and sensor degradation on target detection were investigated.

Numerous elements of the electronics and electrode design that affect the signal-to-noise ratio of the system were investigated. Of particular interest were various modulation techniques. Also, drift and sensitivity were

studied among different identically-treated electrodes for background subtraction and *in situ* calibration. This work was critical in determining the design of the front-end electronics of the high-speed signal processing system specifically for use with a multi-element sensor array using a high throughput field-programmable gate array (FPGA). This A/D system can simultaneously process signals from multi-element arrays of electrodes while enabling sophisticated self-calibration and background subtraction. The FPGA design will support rapid assays and simultaneous detection of multiple targets.

CONCLUSIONS

A breadboard system has been developed that enables testing of the eDNA sensor. The design demonstrates a low cost, low power, lightweight instrument with a long shelf-life that has a reusable sensor and is well-suited for biohazard detection. The biochip has been developed to the breadboard level and has demonstrated the following benefits: capable of detecting to multiple analytes, tailored for CBD and/or food safety, sequence-specific and label-free, free of cumbersome sample preparation, highly sensitive and selective, free of optical components, free of high voltages, rugged and hand held. The biochip can be configured as a plug-in disposable to interface with a portable analysis system approximately the size of a palmtop computer or PDA (personal digital assistant). The sensor has been shown to be capable of detecting and discriminating similar targets (H5N1 and H1N1). When fully developed the sensor will be: capable of responding within ~30 min, able to interface to a personal computer or PDA, and designed to sell for ~$2000.

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23. Due Date: 09/22/2008

24. Conference is ☒ Open to general public ☐ Unclassified/controlled access ☐ Classified

25. For nonpublic meetings: Will foreign nationals be attending?
☐ No ☐ Yes *(If yes, list countries and identify International Agreement(s))*
☐ Don't know

26. Material will be
☐ Oral presented only ☒ Oral presented and published in
Army Science Conference Proceedings
(If published, complete block 18 and 19, Section C.)

E. Authors Statement: 27. All authors have concurred in the technical content and the sequence of authors. All authors have made a substantial contribution to the manuscript and all authors who have made a substantial contribution are identified in Block 3.

James J. Sumner — ARL Lead Author or COR James J. Sumner — 9/9/2008 (Date)

F. Approvals

28. First line Supervisor of Senior ARL Author or COR
Nicholas Fell — Name Nicholas Fell — 09Sep08 (Date)

29. Reviewer(s) *(Technical/Editorial/NA)*
Dimitra Stratis-Cullum — Name(s) Dimitra Stratis-Cullum — 9/10/2008 (Date)

30. Limited distribution information for release to foreign nationals

Foreign Disclosure — Date

31. Classified Information
Classified by
Declassified on

Command Security Manager — Date

ARL Form 1
October 2003

OPSEC REVIEW CHECKLIST

OPSEC POC: Complete and explain any positive responses in block 9.
Note: ARL must be the proponent of the proposed information for release.

1. Does this material contain Sensitive Information? ☐ YES ☑ NO

2. Does this information contain state-of-the-art, breakthrough technology? ☐ YES ☑ NO

3. Does the United States hold a significant lead time in this technology? ☐ YES ☑ NO

4. Does this information reveal aspects of reverse engineering? ☐ YES ☑ NO

5. Does this material reveal any security practices or procedures? ☐ YES ☑ NO

6. Does this information reveal any security practices or procedures? ☐ YES ☑ NO

7. Would release of this information be of economic benefit to a foreign entity, adversary, or allow for the development of countermeasures to the system or technology? ☐ YES ☑ NO

8. Does this material contain:

a. Any contract proposals, bids, and/or proprietary information? ☐ YES ☑ NO

b. Any information on inventions/patent application for which patent secrecy orders have been issued? ☐ YES ☑ NO

c. Any weapon systems/component test results? ☐ YES ☑ NO

d. Any ARL-originated studies or after action reports containing advice and recommendations? ☐ YES ☑ NO

e. Weakness and/or vulnerability information? ☐ YES ☑ NO

f. Any information on countermeasures? ☐ YES ☑ NO

g. Any fielding/test schedule information? ☐ YES ☑ NO

h. Any Force Protection, Homeland Defense *(security)* information? ☐ YES ☑ NO

i. Information on subjects of potential controversy among military services or other federal agencies? ☐ YES ☑ NO

j. Information on military applications in space, nuclear chemical or biological efforts: high energy laser information; particle beam technology; etc? ☐ YES ☑ NO

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OPSEC Approval Statement

I, the undersigned, am aware of the adversary's interest in DOD publications and in the subject matter of this material and that, to the best of my knowledge, the net benefit of this release out weights the potential damage to the essential security of all ARL, AMC, Army, or other DOD programs of which I am aware.

Doran Smith — Doran Smith — 10 Sept 08

OPSEC Reviewer *(Printed name/signature)* — Date

9. Space for explanations/continuations/OPSEC review comments

Final Release Clearances

32. Public/Limited release information

a. Material has been reviewed for OPSEC policy.

ARL OPSEC Officer — SEP 12 2008 — Date

b. The information contained in this material is ☐ / is not ☐ approved for public release/ has received appropriate tech/editorial review.

for GARY L. WOOD — Division Chief — 17 Sep 2008 — Date

c. This information is accepted for public release.

Public Affairs Office — Date